

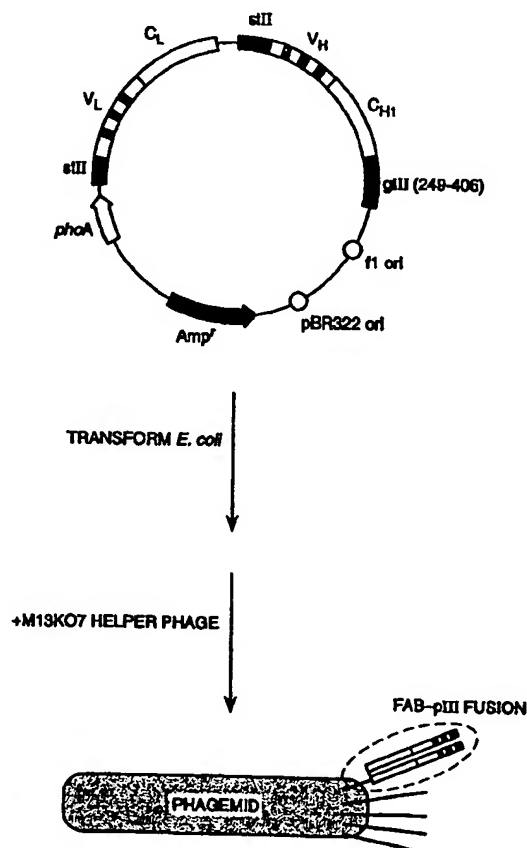


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|-----------|--|
| (51) International Patent Classification ⁶ : C07K 16/22, C12N 15/13, 15/63, 15/70, A61K 39/395 | A3 | (11) International Publication Number: WO 98/45332 (43) International Publication Date: 15 October 1998 (15.10.98) |
| (21) International Application Number: PCT/US98/06724 (22) International Filing Date: 3 April 1998 (03.04.98) (30) Priority Data: 08/833,504 7 April 1997 (07.04.97) US (71) Applicant (for all designated States except US): GENENTECH, INC. [US/US]; One DNA Way, South San Francisco, CA 94080 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): WELLS, James, A. [US/US]; 1341 Columbus Avenue, Burlingame, CA 94010 (US). BACA, Manuel [AU/US]; Apartment #H3, 888 Foster City Boulevard, Foster City, CA 94404 (US). PRESTA, Leonard, G. [US/US]; Apartment 206, 1900 Gough, San Francisco, CA 94109 (US). (74) Agents: DREGER, Walter, H. et al.; Flehr, Hohbach, Test, Albritton & Herbert LLP, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 3 December 1998 (03.12.98) |

(54) Title: HUMANIZED ANTIBODIES AND METHODS FOR FORMING HUMANIZED ANTIBODIES**(57) Abstract**

Described herein is a humanized antibody to vascular endothelial growth factor (VEGF). Also described herein is a method for rapidly producing and identifying framework mutations which improve the binding of humanized antibodies to their cognate antigens. In a preferred embodiment, non-human CDRs are grafted onto a human V_LκI-V_HIII framework. Random mutagenesis of a small set of critical framework residues is also performed followed by monovalent display of the resultant library of antibody molecules on the surface of filamentous phage. The optimal framework sequences are then identified by affinity-based selection. Optionally, the selected antibodies can be further mutated so as to replace verrier residues which sit at the V_L-V_H interface by residues which match the non-human parent antibody. The methods described herein can be applied to any non-human antibody. Accordingly, humanized antibodies are provided.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakhstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/06724

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K16/22 C12N15/13 C12N15/63 C12N15/70 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| X | WO 92 22653 A (GENENTECH INC) 23 December 1992 | 3, 11 |
| Y | the whole document and specially: see SEQ.ID.N. 17 and 18 see page 5, line 24 - page 7, line 35 see page 9, line 22 - page 10, line 4; figure 5 | 1, 2, 9, 10 |
| Y | KIM ET AL.,: "Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo" NATURE, vol. 362, 1993, page 841 XP002013864 London, GB cited in the application see abstract | 1, 2, 9, 10 |
| | --- -/-- | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

18 September 1998

Date of mailing of the international search report

02/10/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Mateo Rosell, A.M.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/06724

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| A | US 5 580 723 A (WELLS JAMES A ET AL) 3 December 1996 see figures 12A-J --- | 19,20 |
| A | WO 94 04679 A (GENENTECH INC) 3 March 1994 see page 1-73 --- | 1,9 |
| A | GB 2 268 744 A (CELLTECH LTD) 19 January 1994 see abstract see page 4, paragraph 3 - page 6, paragraph 1 --- | 1,9 |
| A | M.M. BENDIG: "Humanization of rodent monoclonal antibodies" METHODS: A COMPANION TO METHODS IN ENZYMOLGY, vol. 8, 1995, pages 83-93, XP000647344 New York, NY, US see the whole document --- | 1,10 |
| P,X | M. BACA ET AL., : "Antibody humanization using monovalent phage display" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 16, 18 April 1997, pages 10678-10684, XP002077471 see the whole document ----- | 1-14 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/06724

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO 9222653 A | 23-12-1992 | AU 675916 B | 27-02-1997 |
| | | AU 2250992 A | 12-01-1993 |
| | | CA 2103059 A | 15-12-1992 |
| | | EP 0590058 A | 06-04-1994 |
| | | JP 6508267 T | 22-09-1994 |
| | | WO 9404679 A | 03-03-1994 |
| US 5580723 A | 03-12-1996 | US 5766854 A | 16-06-1998 |
| | | US 5534617 A | 09-07-1996 |
| | | EP 0397834 A | 22-11-1990 |
| | | JP 4502454 T | 07-05-1992 |
| | | WO 9004788 A | 03-05-1990 |
| | | CA 2001774 A | 28-04-1990 |
| WO 9404679 A | 03-03-1994 | US 5688666 A | 18-11-1997 |
| | | AU 675916 B | 27-02-1997 |
| | | AU 2250992 A | 12-01-1993 |
| | | EP 0590058 A | 06-04-1994 |
| | | JP 6508267 T | 22-09-1994 |
| | | CA 2103059 A | 15-12-1992 |
| GB 2268744 A | 19-01-1994 | WO 9222653 A | 23-12-1992 |
| | | AU 5083193 A | 15-03-1994 |
| | | AT 129017 T | 15-10-1995 |
| | | AT 124459 T | 15-07-1995 |
| | | AT 159299 T | 15-11-1997 |
| | | AU 664801 B | 30-11-1995 |
| GB 2268744 A | 19-01-1994 | AU 6461294 A | 22-12-1994 |
| | | AU 646009 B | 03-02-1994 |
| | | AU 6974091 A | 24-07-1991 |
| | | AU 649645 B | 02-06-1994 |
| | | AU 7033091 A | 24-07-1991 |
| | | AU 631481 B | 26-11-1992 |
| | | AU 7048691 A | 24-07-1991 |
| | | BG 60462 B | 28-04-1995 |
| | | CA 2037607 A | 07-09-1992 |
| | | CA 2046904 A | 22-06-1991 |
| | | CA 2050479 A,C | 22-06-1991 |
| | | DE 69020544 D | 03-08-1995 |
| | | DE 69020544 T | 18-01-1996 |

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 98/06724

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| GB 2268744 A | | DE 69022982 D | 16-11-1995 |
| | | DE 69022982 T | 28-03-1996 |
| | | DE 69031591 D | 20-11-1997 |
| | | DE 69031591 T | 12-03-1998 |
| | | DK 460167 T | 20-11-1995 |
| | | DK 460171 T | 28-08-1995 |
| | | DK 460178 T | 22-12-1997 |
| | | EP 0460167 A | 11-12-1991 |
| | | EP 0460171 A | 11-12-1991 |
| | | EP 0460178 A | 11-12-1991 |
| | | EP 0620276 A | 19-10-1994 |
| | | EP 0626390 A | 30-11-1994 |
| | | ES 2079638 T | 16-01-1996 |
| | | ES 2074701 T | 16-09-1995 |
| | | ES 2112270 T | 01-04-1998 |
| | | WO 9109966 A | 11-07-1991 |
| | | WO 9109967 A | 11-07-1991 |
| | | WO 9109968 A | 11-07-1991 |
| | | GB 2246781 A,B | 12-02-1992 |
| | | GB 2246570 A,B | 05-02-1992 |
| | | GB 2268745 A,B | 19-01-1994 |
| | | GR 3017734 T | 31-01-1996 |
| | | GR 3025781 T | 31-03-1998 |
| | | JP 4505398 T | 24-09-1992 |
| | | JP 4506458 T | 12-11-1992 |
| | | JP 5500312 T | 28-01-1993 |